

The University of Chicago

STUDIES ON THE BACTERIOLOGY AND IMMUNOL-
OGY OF CHRONIC STAPHYLOCOCCAL OSTEO-
MYELITIS: I. THE CULTURES INVOLVED: THE
ANTIHEMOLYSIN IN THE PATIENT'S SERUM:
AND THE LOCAL INFLAMMATORY REACTION

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY

1938

By
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CHICAGO, ILLINOIS

Reprinted from
THE JOURNAL OF INFECTIOUS DISEASES
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STUDIES ON THE BACTERIOLOGY AND IMMUNOLOGY OF CHRONIC STAPHYLOCOCCAL OSTEOMYELITIS: I. THE CULTURES INVOLVED: THE ANTIHEMOLYSIN IN THE PATIENT'S SERUM: AND THE LOCAL INFLAM- MATORY REACTION

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Recent studies on staphylococci in infections have been concerned chiefly with toxin-antitoxin relationships, and little consideration has been given to reactions of the host. Numerous investigators have shown that staphylococcus toxin is important in generalized toxemia and septicemia due to these organisms, and marked alleviation of symptoms has been reported in such cases as a result of therapies designed to elevate the humoral antibodies of the patient.¹ Favorable results, however, have not been generally obtained by use of such therapeutic measures in chronic infections where toxic symptoms are minor or absent. Buchman² and Blair and Hallman³ found elevation of humoral antihemolysin in patients with chronic osteomyelitis after toxoid immunization, but they failed to find a concurrent clinical improvement of the patient. Joyner and Smith⁴ rightfully considered the development of chronic draining sinuses an indication of improvement in patients with acute osteomyelitis treated with toxoid. However, healing of the chronic lesions was not shown to be fostered by this treatment.

Recently Forssman⁵ and Smith⁶ demonstrated the development of local abscesses in immunized rabbits following injection of staphylococci. The results of these investigations imply that toxin-antitoxin relationships are not as important to local lesion production and treatment as formerly believed.

Patients with chronic osteomyelitis not artificially immunized show little or no increase in antihemolysin except during or immediately following acute exacerbation.³ Yet, staphylococci are present in large numbers in the chronic draining lesions.

Marchand⁷ suggested that chronic fibrous inflammations and fibrin de-

Received for publication, February 12, 1938.

1. Burnet, F. M.: *J. Path. & Bact.* **32**: 717, 1929; Murray, D. S.: *Lancet* **1**: 303, 1935; Dolman, C. E.: *J.A.M.A.* **100**: 1007, 1933, and *Lancet* **1**: 306, 1935; Ranom, C., Bocage, A., Richou, R. and Mercier, P.: *Presse méd.* **44**: 185, 1936; Parish, H. J., O'Meara, R. A. Q. and Clark, W. H. M.: *Lancet* **1**: 1054, 1934; Whitby, L. E. H.: *Lancet* **1**: 1454, 1936.

2. *J.A.M.A.* **108**: 1151, 1937

3. *Proc. Soc. Exper. Biol. & Med.* **34**: 637, 1936.

4. *Surg. Gynec. & Obst.* **63**: 1, 1936.

5. *Acta path. et microbiol. Scandinav.* **13**: 453, 1936.

6. *J. Path. & Bact.* **45**: 305, 1937.

7. *Handb. d. allgem. Path.* **4**: 255, 1924.

posits on serous membranes might be inhibiting factors to dissemination of bacteria or their products from the site. Recent researches lend credence to this belief. Fox⁸ has shown in rabbits that various materials injected intravenously were excluded from inflammatory areas over 24 hours old. This author reported that blood-vascular elements were reduced in the areas of chronic inflammation, and considered this to be an important factor to the impermeability of the lesions. While this work suggests that substances within the lesion would be excluded from the general circulation, this point has not been demonstrated directly, nor has impermeability of the inflammatory lesion been accredited significance to disease processes of man.

In view of the work just discussed it appeared that a study of the host-parasite relationships in chronic osteomyelitis might be fruitful. From this point of view we have studied: (1) toxin-production and other biological characteristics of strains of staphylococci isolated from lesions of human osteomyelitis; (2) the antihemolysin titer of the patient's serum over a long

TABLE 1.—*Reactions Characterizing Strains of Staphylococci*

Reaction	Osteomyelitis Strains		Other Strains		Total Number Tested
	Positive	Negative	Positive	Negative	
Mannite fermentation	78	7	12	0	97
Lactose fermentation	78	5	9	0	92
Gelatin liquefaction	83	2	11	1	97
Coagulase production	37	56	12	4	109

period of time and in response to toxoid injections; (3) the dissemination of antigens and antibodies when introduced into inflammatory sinus tracts of patients and experimental animals; and (4) the in vitro effect of cells from pleural exudate of rabbits on staphylococcus toxin and other test substances used in these experiments.

STUDIES ON STAPHYLOCOCCI ISOLATED FROM CASES OF OSTEOMYELITIS

Pathogenic staphylococci characteristically ferment numerous carbohydrates such as lactose, mannite and glucose, liquefy gelatin, hydrolyze peptone, produce coagulase and fibrinolysin, produce toxins and induce disease in animals, while non-pathogenic strains usually fail to give these reactions.⁹ We have determined the ability to ferment mannite and lactose, liquefy gelatin and produce coagulase of cultures isolated from osteomyelitis. Several toxigenic strains from other sources were included for purposes of comparison. Either agar or broth containing the test carbohydrate with brom-cresol-purple as an indicator was used in the fermentation reactions. Coagulase tests were made in 10% oxalated rabbit plasma in saline, which was inoculated with a loopful of organisms from an 18–24 hour agar slant culture. If coagulation did not occur within three hours incubation the strain

8. J. Immunol. **31**: 293, 1936.

9. Julianelle, L. A. and Wieghard, C. W.: J. Exper. Med. **62**: 11, 1935; Thompson, R. and Khorazo, D.: J. Bact. **32**: 39, 1937, and **34**: 1, 1937; Stritar, J. and Jordan, E. O.: J. Infect. Dis. **56**: 1, 1935; Chinn, Ben D.: Food Research **1**: 513, 1936; Chapman, C. H., Berens, C., Peters, A. and Curcio, L.: J. Bact. **28**: 343, 1934; McBroom, J.: J. Infect. Dis. **60**: 364, 1937.

was considered to be negative. This is a more rigid method than that often used for detection of coagulase. Results of these tests are summarized in table 1.

Osteomyelitis strains did not differ from the pathogenic strains from other sources in the fermentation of mannite and lactose and the liquefaction of gelatin. They were less active in the production of coagulase (39.8% of osteomyelitis strains positive). The inability of many strains to produce coagulase should not exclude them from being classified with the pathogenic staphylococci.

The production of toxin.—A study was made of toxin-production of osteomyelitis strains by assaying Berkefeld filtrates for alpha and beta hemolysin and dermo-necrotoxin. The minimal hemolytic unit and minimal dermo-necrotizing unit were employed. Toxins were prepared in small amounts from extracts of semi-solid agar cultures grown in an atmosphere of 20% CO₂. The titer obtained using the small volume was less than that

TABLE 2.—*Toxin Production by Strains of Staphylococci*

Number of Determinations—123 Number of Strains Tested — 93		Osteomyelitis Strains —74 Strains from other Sources—19	
Toxin Tested	Median	Mean	Mode
Alpha hemolysin (rabbit cell) MHD			
Osteomyelitis	0.1 cc.	0.26 cc.	0.5 and 0.05 cc.
Others	0.05	0.029	0.025
Beta hemolysin (sheep cell) MHD			
Osteomyelitis	0.5 cc. (neg.)	0.14 cc.	0.5 cc. (neg.)
Others	0.5	0.384	0.5
Dermo-necrotoxin MD-ND			
Osteomyelitis	0.0025 cc.	0.0080 cc.	0.0 and 0.0025 cc.
Others	0.0025	0.00195	0.0025 and 0.00125

MHD = minimal hemolytic dose.

MD-ND = minimal dermo-necrotizing dose.

produced by the same strains when larger amounts were prepared, probably due to absorption of toxin on the filters. Even though maximum titers were not obtained using the small volume of filtrate, the comparative value is of significance since uniform technic was employed.

We have assayed 123 filtrates of 93 strains, 74 of which were isolated from osteomyelitis. The remaining strains were from other sources, and most of them were toxin-producers, included for purposes of comparison. Single determinations were made on osteomyelitis strains, multiple tests being performed on two of the strains used for comparison (Wood no. 43 and no. 8, a food poisoning organism isolated in this laboratory). Statistical calculations were made to simplify the mass of crude data. The mean (arithmetical average), the median (the middle figure when all values were arranged in numerical order), and the mode (most frequently appearing value), were found for each of the three reactions studied. These statistical values are recorded in table 2.

The mean value for toxin production by osteomyelitis strains was found to be somewhat less than that for the other cultures. However, the latter group was composed largely of selected toxin-producing organisms. Exami-

nation of the mode table 2, reveals that the osteomyelitis strains fell into 2 groups, one of which produced little or no toxin, while toxin of the other group was of a potency comparable to that produced by the control group. The general experience has been that ability of a strain to produce toxin bears little relationship to its source, and it would appear from this work that osteomyelitis cultures do not differ in this respect from strains isolated from other disease processes.

In 10 instances 2 or more strains from the same patient were available for study. The titers of filtrates of these strains are listed in table 3. In two cases (patients 2 and 3), mixed cultures were found. The "A" strain from these two patients was in each case *Staph. aureus* and the "B" strain was *Staph. albus*. Other cultures were isolated from foci during recurrence of the infection. In all cases except 3 (patients 2, 3 and 1), the titers of the toxins were strikingly similar. Inference that this similarity of toxin potency would necessarily indicate identity of the strains is not intended.

TABLE 3.—*Toxin Production by Strains of Staphylococci from Osteomyelitis Lesions in the Same Patient*

Patient	Strain	Hemolysins (MHD)		Dermo-necrotoxin (MD-ND)
		Alpha	Beta	
1	2A	0.5	neg.	0.005
	2B	neg.	neg.	neg.
2	3A	0.05	0.5	0.00125
	3B*	neg.	neg.	neg.
3	9A	0.025	neg.	0.00062
	9B*	0.05	neg.	neg.
4	40	0.1	0.5	0.00125
	41	0.1	0.5	0.00125
5	44	0.05	neg.	neg.
	44B	0.5	neg.	neg.
6	45	0.05	0.5	neg.
	45B	0.05	neg.	neg.
7	45C	0.5	neg.	neg.
	47	0.5	neg.	0.005
8	47B	neg.	neg.	0.005
	32	0.5	neg.	0.000125
9	32B	0.05	neg.	0.00125
	55	0.1	0.5	0.0025
10	55B	0.5	neg.	0.005
	25	0.05	neg.	0.00125
	25B	0.025	0.5	0.00031

* These were albus strains isolated at the same time.

RELATION OF TOXICITY TO DISEASE PRODUCTION IN NORMAL AND IMMUNE ANIMALS

The relation of toxicity and coagulase production of the culture¹⁰ and the immunity^{5,6} of the animal to the type of disease produced in rabbits has been studied by several workers. We inoculated rabbits subcutaneously with 2.5 cc. of broth cultures of different strains of staphylococci, the in vitro toxicity of which had been previously determined. Some of the animals had been immunized with toxoid and toxin. We noted the type of lesion produced by each of the strains and attempted to correlate this with the immunity of the animal and with ability of the strain to produce toxin and coagulase. Data are given in table 4.

10. Menkin, Valy, and Walston, H. D.: *Proc. Soc. Exper. Biol. & Med.* **32**: 1259, 1935.

Individual variation among rabbits was marked, but certain general trends were noted. Immunized animals developed lesions which were essentially similar to those of normal animals injected with the same culture. Injection of strain 9B, which produced coagulase but no hemolysin or dermo-necrotoxin, induced spreading lesions that differed greatly from the others in extent of involvement, amount of necrosis and time of development. This culture was the most invasive of the group which we tested. Localized lesions resulted from inoculation with either toxin-producers or strains completely negative in this respect.

TABLE 4.—*Local Lesion Production Following Subcutaneous Inoculation by Strains of Known Toxin Production**

Rabbit	Culture	Toxic Properties		Coagulase	Lesion
		Hemolysins			
		Alpha	Beta	D-N	
1	43	±	+	+	Slight erythema followed by induration.
2	2A	±	0	+	Diffuse redness; slight induration.
3	1	+	0	0	Redness & induration followed by small abscess; animal emaciated; diarrhea.
4					Redness followed by small pustule.
5					Redness and induration; animal died; cause undetermined.
6					Well localized abscess with elevated soft margins; 3.5 × 1.5 cm.
immune					Redness & induration; 2 × 3 cm.; pus present.
7					
immune					
8	24	0	0	0	No reaction.
9					Induration and redness; 3 × 0.5 cm.
10					Lesion 3 × 1.5 cm.; later slight papule.
11					Diffuse redness followed by small abscess.
12	33	+	0	+	Slight diffuse redness.
13					No reaction.
14					Redness and induration; 5 × 0.5 cm.; short duration.
15					Induration and redness followed by a small pustule.
16	28	0	0	0	Redness and edema after 24 hrs.; no reaction at 3 days.
17					Redness and edema at 24 hrs. followed by small pustule; rapidly disappeared.
18	9B	0	0	0	Lesion 10 × 4 cm. extending to linea alba; much necrosis; animal died in 48 hours.
19					Induration and redness 6 cm. diameter; central necrotic area.
20					Lesion diffuse hemorrhagic and necrotic in 24 hrs. extending to linea alba with involvement of leg; sloughed and healed.
21					Induration, redness and necrosis 6 × 3 cm.
immune					
22					Red and indurated, and necrotic 7 × 4 cm.
23					Red and indurated 6 cm. diameter; necrosis 0.5 c. diameter.
immune					

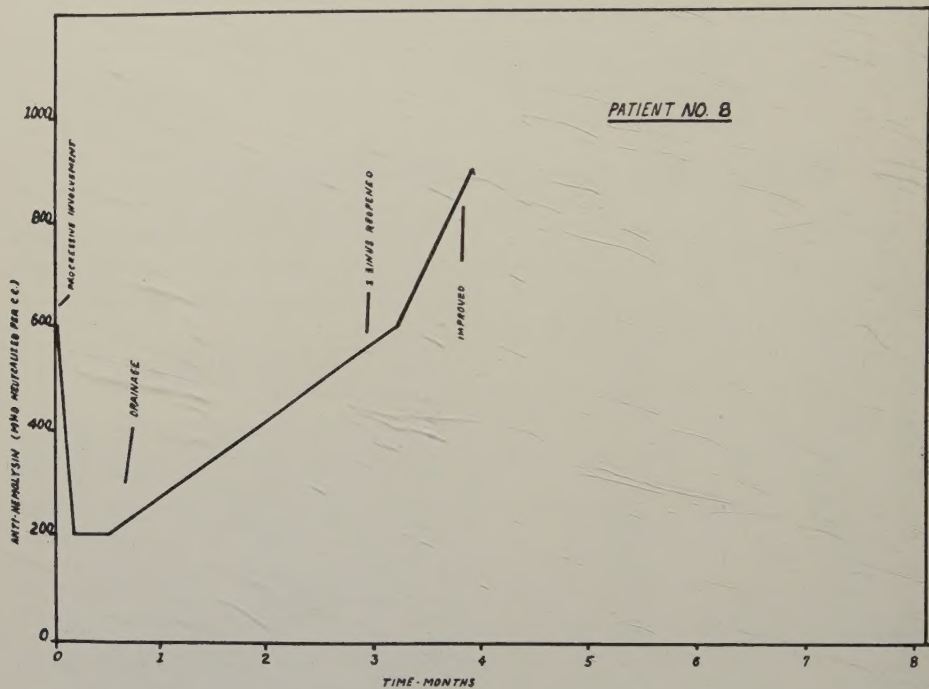
* 2.5 cc. of culture were injected into each rabbit.

On the basis of these experiments we feel justified in concluding with Forssman⁵ and Smith⁶ that antitoxic immunity of the animal bears no relationship to the development of localized lesions, and with Menkin and Walston¹⁰ that coagulase is inactive in the local "walling-off" or fixation of the inflammatory process. Our results would indicate that some property of staphylococci other than toxicity or coagulase production was important in the production of localized lesions.

ANTIHEMOLYSIN TITER OF PATIENT'S SERUM

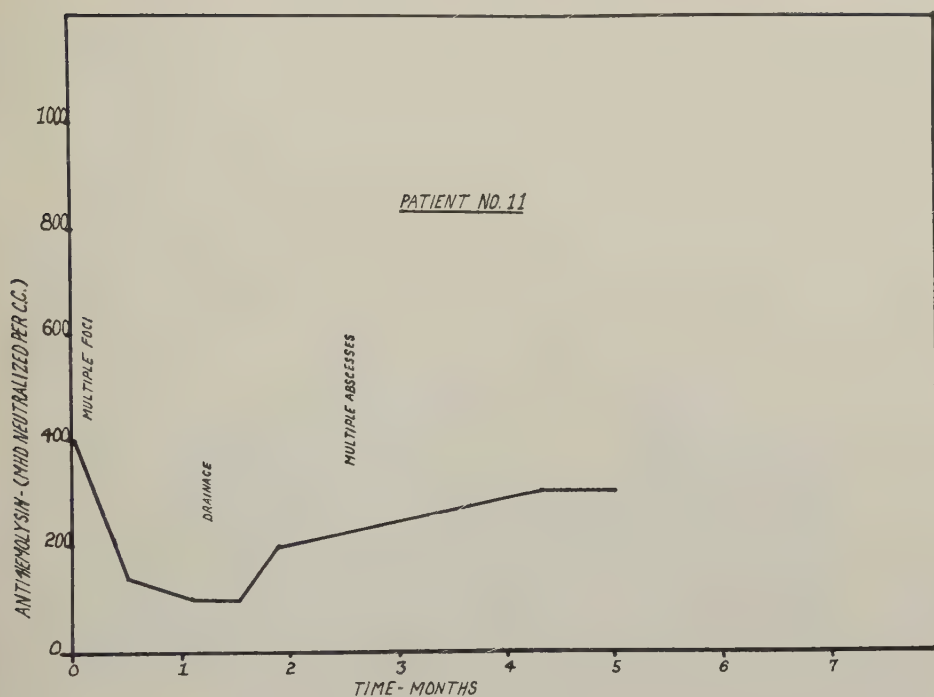
The antihemolysin titer of the serum of 23 patients was followed for periods from 3 months to over a year and determinations were made on single samples from fourteen additional patients. We have not been interested in

establishing any absolute values for antihemolysin in the serum but have correlated the titer with the clinical condition of the patient. The curves obtained by plotting the antihemolysin values against time are given for four patients (graphs 1-4). The clinical condition of the patient is indicated on the graph. Two of the graphs are of patients that received toxoid injections; the others received no such treatment. Data for each of the 23 patients are available and are similar in all respects to the ones presented here. We are indebted to Dr. Dallas B. Phemister for the opportunity to study these patients, and to Dr. Paul H. Harmon for the immunization of patients with toxoid.

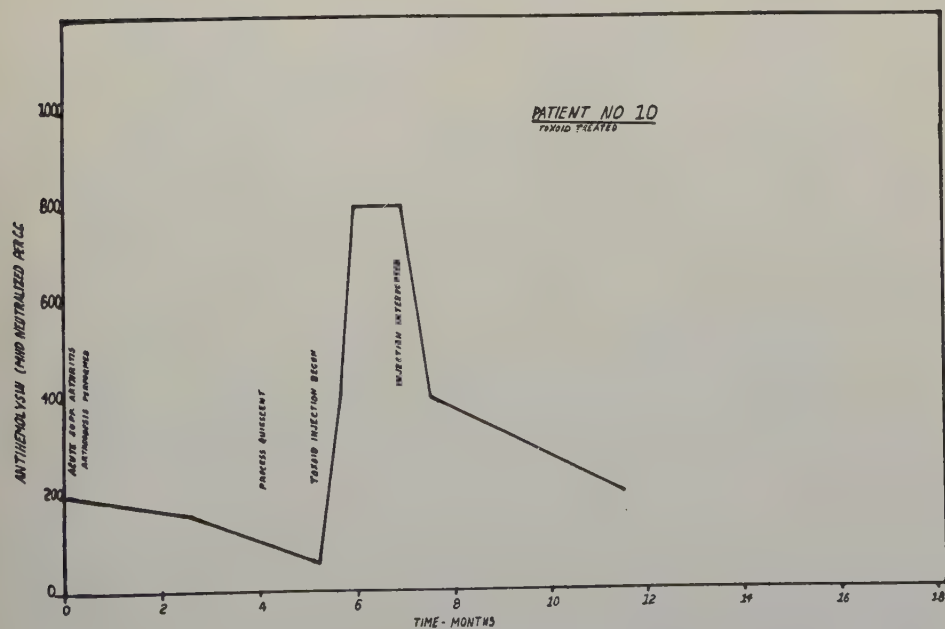


Graph 1.—Antihemolysin titer of patient 8.

A positive correlation was found in all cases between presence of active disease and elevated antihemolysin. The titer remained elevated so long as progressive involvement continued and rapidly became reduced during periods of quiescence and drainage. Elevated humoral antihemolysin did not seem to prevent development of multiple foci and numerous soft tissue abscesses during an acute exacerbation. It therefore seems that development of local lesions in patients during one attack is similar to that in immunized animals, and is not inhibited by high circulating antihemolysin. In this series of patients, however, it was apparent that recurrence of the infection at a later date followed a period during which the titer was low. That is, progressive involvement and development of multiple foci of infection may represent development of lesions in sites in which the organisms have become localized at about the time of development of the first lesion of the exacerbation when



Graph 2.—Antihemolysin titer of patient 11.

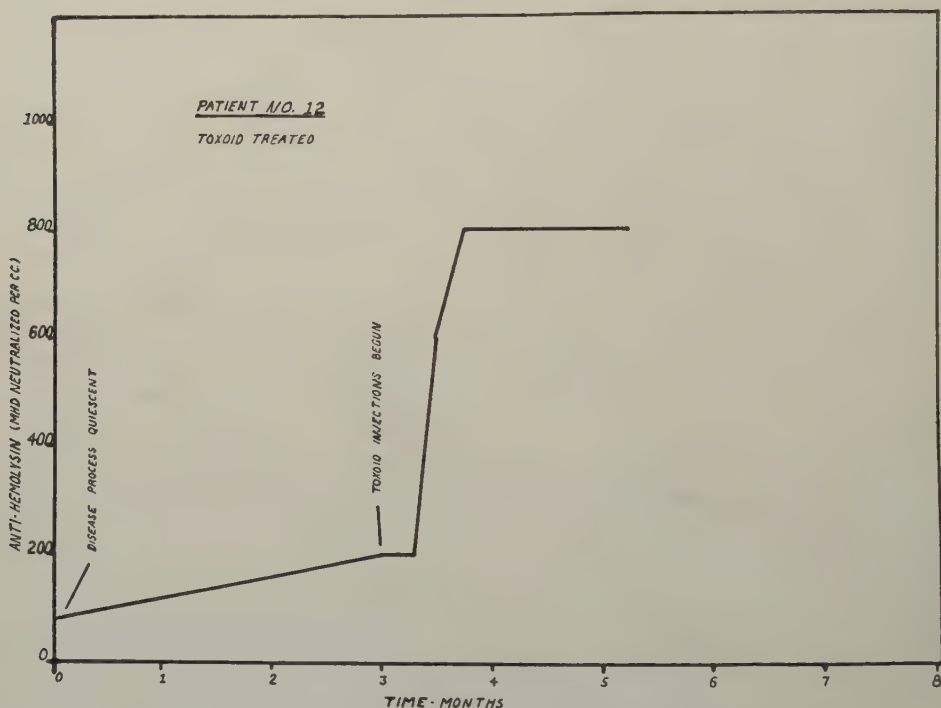


Graph 3.—Antihemolysin titer of patient 10, toxoid treated.

the antihemolysin titer was low. Recurrence would thus represent a re-enactment of these conditions. In support of this view is the common clinical observation that a septicemia is often detectable for a short time at the beginning of an acute exacerbation.

DISSEMINATION OF ANTIGENS AND ANTIBODIES FROM INFLAMMATORY SINUS TRACTS

The low antihemolysis titer of the patient's serum during the chronic stages of osteomyelitis suggested that the chronic inflammation of the sinus wall exerted an effect on the exchange of materials between the sinus and the



Graph 4.—Antihemolysin titer of patient 12, toxoid treated.

circulation, and that such a condition would account, at least in part, for the general responses of the patient to the infection.

This possibility was tested both in patients and in rabbits in which experimental inflammatory sinuses were prepared. In patients the method consisted of introducing concentrated typhoid vaccine into draining sinuses and testing the serum for the presence of agglutinins against *Bact. typhosum*. The vaccine used in these and the animal experiments contained 18 billion organisms per cc. Each patient received 1.2 cc. given in 3 applications. The vaccine was applied to the gauze packing placed in the sinuses from which drainage was slight. Four patients have been tested so far, and no increase in typhoid agglutinins occurred.

Experimental sinus tracts were produced in rabbits by embedding rubber

catheters subcutaneously. The catheter was anchored within the tissue by means of a bent platinum or glass rod fastened to its tip and the other end was brought to the outside and stitched to the skin. This method was ad-

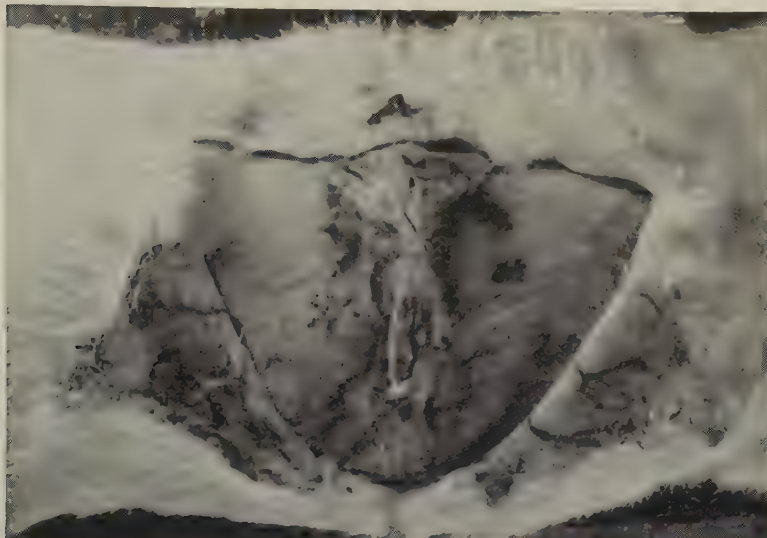


Fig. 1.—Rabbit 37—Showing subcutaneous inflammatory reaction surrounding catheter.

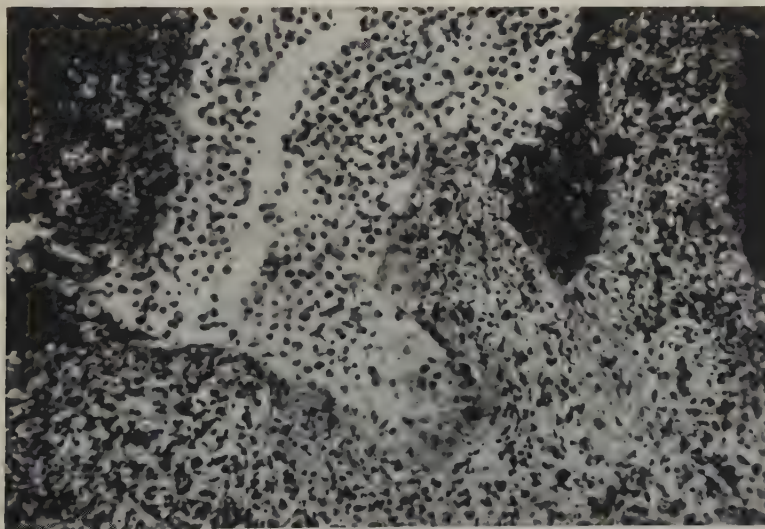


Fig. 2.—Photomicrograph of sinus wall of rabbit 42.

vantageous in that materials could be introduced into the lumen without penetrating the wall of the sinus. Fig. 1 is a photograph of one of these preparations. The skin has been reflected to show the subjacent inflammation. At the conclusion of each experiment gross examination was made and tissue was taken for histological study. Fig. 2 is a photomicrograph of a section

through the sinus wall of rabbit 42. The reaction in each case was of the chronic fibrous type. Exudate cells were chiefly polymorphonuclear leucocytes. These cells as well as large numbers of macrophages and fibroblasts were present in the sinus wall. A marked fibrous layer was constantly seen near the central margin of the sinus wall in microscopic preparations. The amount of pus present in the tracts was estimated at 3–10 cc. and differed with the age of the inflammation and in individual rabbits. The blood vessel relationships were particularly interesting. Proliferation had occurred around the periphery, but there was little evidence of penetration of vessels into the

TABLE 5.—*Development of Humoral Antibodies Following Injection of Antigens into Experimental Sinus Tracts in Rabbits*

Rabbit	Duration of Sinus Tract (Days)	Agglutinins for Bact. typhosum in Serum		Horse Plasma Precipitins*
		Control Serum	Titer After Injection of Antigen	
24	0	1:40	1:800	+1:10,000
25	0	1:20	1:400	0
26	0	1:10	1:1,600	1:10,000
27	0	1:20	1:1,600	+1:10,000
28	0	1:80	1:1,600	+1:10,000
29	0	1:20	1:800	1:1,000
30	0	1:20	1:3,200	1:1,000
31	1	1:20	1:400	1:100
32	1	1:10	1:100	0
33	1	1:40	1:200	1:100
34	1	1:20	1:100	0
35	1	0	1:160	0
36	1	1:80	1:160	0
37	1	1:40	1:160	1:1,000
38	1	1:20	1:320	1:10,000
39	1	1:20	1:320	0
40	1	1:40	1:160	+1:10,000
41	10	1:20	1:400	1:1,000
42	10	1:40	1:300	0
43	10	1:40	1:200	1:1,000
44	10	1:80	1:160	0
45	10	1:80	1:80	0
46	10	1:20	1:160	0
47	10	1:20	1:80	1:100
48	10	1:80	1:160	0
49	10	1:40	1:320	0
50	10	1:20	1:80	0

* No precipitins were found in the serums taken before introduction of the antigen. 0 indicates negative results.

center of the area. When the peripheral tissue containing the larger blood vessels was stripped from the underlying portion of the lesion almost no seepage occurred, and only a few capillaries were seen in the central portions of the sinus wall in microscopic preparations.

A mixture of horse plasma and typhoid vaccine was injected through the catheters of ten rabbits 24 hours after operation. Ten others received similar treatment 10 days after the operation. Control animals were inoculated subcutaneously with a mixture of these substances in combination with a 24 hour broth culture of staphylococci. All controls and a few experimental animals received 0.3 cc. of typhoid vaccine and 0.4 cc. of horse plasma. Other animals received 0.5 cc. of each of these materials. Leakage around the catheters was slight. All animals were bled immediately before and 7–10 days after introduction of the antigens. Serums were tested for agglutinins for Bact. typhosum and precipitins for horse plasma. Titers are given in table 5.

Agglutinins were increased in the majority of animals as a result of introduction of the vaccine, but the titers were in most cases lower than those of the controls. Only 9 animals developed precipitins against horse plasma. The occurrence of significantly elevated titers against both these antigens was certainly more consistent in the controls.

At the conclusion of the experiments 0.5 cc. of botulinum antitoxin (2,000 units per cc.) was injected into the sinuses of the 11 animals that had retained them. Control animals were inoculated subcutaneously. Twenty-four hours later 1.0 cc. of a 1:10 dilution of botulinum toxin was given intravenously. Only the control animals survived the test dose of toxin. The protocol is given in table 6.

TABLE 6.—*Absorption of Botulinum Antitoxin from Experimental Sinus Tracts in Rabbits*

Duration of Sinus Tract (Days)	Number of Animals Tested	Mode of Injection of Antitoxin*	Survival Following Intravenous Injection of Botulinum Toxin
7	1	Sinus	0
9	6	Sinus	0
17	2	Sinus	0
21	2	Sinus	0
21	1	Subcutaneous near sinus	0
0	3	Subcutaneous	3**
0	6	0	0

* 0.5 cc. of antitoxin (quoted 2,000 units per cc.) was injected.

** One of these rabbits died after 3 days from a dose of toxin otherwise fatal in 3-4 hours.

EFFECT OF EXUDATE CELLS UPON ANTIGENS AND ANTIBODIES

Pleural exudate from two rabbits was collected 24 hours after the injection of aleuronat solution (Gay and Clark¹¹). The exudate cells were separated by gentle centrifugalization. Two cc. each of staphylococcus toxin, horse plasma and typhoid vaccine were mixed with cells from one-third the exudate obtained from one rabbit. These mixtures were incubated at 37 C. for 2 hours and left in the icebox overnight, after which they were centrifugalized and the supernatant fluid tested for activity. The following results were obtained: the dermo-necrotic and hemolytic properties of staphylococcus toxin were unimpaired, and the typhoid vaccine and horse plasma incited production of antibodies. Cells from the total exudate of one rabbit were used in the treatment of 0.75 cc. of botulinum antitoxin, and this mixture was incubated overnight. The exudate treated antitoxin in 0.25 cc. amounts, protected mice against 0.05 cc. toxin. Under the conditions of the experiment exudate cells were found to have no detrimental effect upon staphylococcus toxin, typhoid vaccine, horse plasma or botulinum antitoxin.

DISCUSSION

Treatment of chronic staphylococcal osteomyelitis with toxoid and antitoxin has been disappointing, although favorable results have been obtained with these agents in acute and generalized infections. In the present study consideration has been given to cultures isolated from lesions, antihemolysin in the patient's serum and the local inflammatory reaction as factors to

explain the clinical observations on the disease process in patients. Study of the fermentation of mannite and lactose, liquefaction of gelatin and production of coagulase revealed no essential difference between osteomyelitis strains and those from other sources. Assay of alpha and beta hemolysin and dermo-necrotoxin in filtrates of 93 strains, 74 of which were isolated from osteomyelitis, showed statistically that osteomyelitis strains could be divided into 2 groups, one of which produced potent toxin, the other little or no toxin. The other strains used in this study were selected as toxin-producing organisms, with which osteomyelitis strains were compared. Differences found between osteomyelitis strains and those used for comparison were not of sufficient magnitude to be considered of major importance to the immunology of the disease. The cultures were in all respects similar to those which have been isolated from other disease processes.

Antihemolysin determinations were made on numerous serum samples collected over long periods of time and during various stages of the disease from 23 patients. In each case the titer was found to rise during or immediately following periods of acute exacerbation, and rapidly fell during the chronic stages. A similar relationship between the antihemolysin titer and the disease process was reflected in the titers of 14 patients from whom only one sample of serum was obtained. Progressive involvement seemed to occur during periods of high titer, but recurrence usually followed a period of reduced antihemolysin. The possibility that progressive involvement represents development of foci to which the organisms have been localized at the beginning of the exacerbation, while recurrence represents a re-enactment of general dissemination and localization, is discussed. Patients given toxoid during the chronic stages of the disease responded with a rapid rise in antihemolysin, but with no demonstrable clinical change.

Local lesions developed following injection of staphylococci in both immunized and normal rabbits. The most spreading and severe lesions were incited by a strain which produced coagulase but no toxin *in vitro*.

These observations would indicate that a similar relation of immunity to development of local lesions is present in both patients and rabbits. Immunity in neither case prevented development of local lesions. Toxicity of strains did not seem to be particularly related to production of local lesions following injection of the organism into rabbits, nor was a relationship between toxicity and involvement in chronic osteomyelitis revealed by study of culture filtrates.

The results of antihemolysin determinations on patient's serum and failure of these patients to respond to toxoid therapy suggested that the chronic inflammatory sinus wall was a barrier to the exchange of materials between the lesion and the circulation. This possibility was tested both in patients and in experimental sinus tracts in the subcutaneous tissue of rabbits. The results indicated that the chronic sinus wall prevented the diffusion of materials introduced into the lumen of the sinus. Typhoid vaccine placed into chronic sinuses of 4 patients resulted in no increase in agglutinins for *Bact. typhosum*. In rabbits typhoid agglutinins and precipitins against horse plasma were irregularly produced when the corresponding antigens were

injected into experimental sinus tracts. In addition, botulinum antitoxin similarly injected failed to protect against intravenous homologous toxin in 11 rabbits.

Incubation with cells from pleural exudate of rabbits did not destroy staphylococcus toxin, botulinum antitoxin or the antigenicity of typhoid vaccine and horse plasma.

SUMMARY

Staphylococci isolated from lesions of patients with chronic osteomyelitis were found to differ in no essential respect from strains isolated from other sources.

Differences between toxin production by osteomyelitis strains and those used for comparison were not of sufficient magnitude to be considered of major importance to the immunology of the disease.

Antihemolysin determinations on numerous samples of patient's serum revealed a relationship between the humoral immunity and the clinical stage of the disease.

Immunity as measured by antihemolysin titer did not prevent the development of local lesions in experimental animals. It is suggested that a similar phenomenon exists in human infections.

The chronic inflammatory sinus tract prevented general dissemination of materials introduced into the lumen, and was, therefore, considered an important factor to the development of general immunity.

In our experience exudate cells exerted no effect on the antigens and antibodies introduced into the sinus tracts.

